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DISINTEGRATION OF PHOSPHATIDYLCHOLINE LIPOSOMES IN PLASMA AS A RESULT OF INTERACTION WITH HIGH-DENSITY LIPOPROTEINS

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Summary

1. During in vitro incubation of liposomes or unilamellar vesicles prepared from egg-yolk or rat-liver phosphatidylcholine with human, monkey or rat plasma the phospholipid becomes associated with a high molecular weight protein-containing component.

2. The phosphatidylcholine · protein complex thus formed co-chromatographs with high-density lipoprotein on Ultrogel AcA34 and has the same immunoelectrophoretic properties as this lipoprotein.

3. Release of phosphatidylcholine from liposomes was also observed when liposomes were incubated with pure monkey high-density lipoproteins. Under those conditions some transfer of protein from the lipoprotein to the liposomes was observed as well.

4. The observed release of phospholipid from the liposomes is a one-way process, as the specific radioactivity of liposome-associated phosphatidylcholine remained constant during incubation with plasma.

5. It is concluded that either the lipoprotein particle takes up additional phospholipid or that a new complex is formed from protein constituents of the lipoprotein and the liposomal phosphatidylcholine.

6. Massive release of entrapped ¹²⁵I-labeled albumin from the liposome during incubation with plasma suggests that the observed release of phosphatidylcholine from the liposomes has a highly destructive influence on the liposomal structure.

7. Our results are discussed with special reference to the use of liposomes as intravenous carriers of drugs and enzymes.

Introduction

Recently we reported on the release of sucrose and inulin from phosphatidylcholine liposomes as induced by rat plasma and bovine serum albumin [1]. Although albumin caused similar leakage rates as whole plasma we had reason to believe that the liposomal phosphatidylcholine, when incubated with whole plasma, was not bound to albumin but rather to a component with higher molecular weight, presumably high-density lipoprotein. In the present paper we provide evidence that single-bilayer vesicles or multilamellar liposomes prepared from phosphatidylcholine are indeed disrupted as a result of interaction in vitro with the high-density lipoprotein fraction of rat, monkey or human plasma. The liposomal lipid becomes associated with a particle which has approximately the same size and electrophoretic mobility as high-density lipoprotein and is precipitated by rabbit antiserum against this lipoprotein. Recently Tall and Small [2] described the disruption of dimyristoyl phosphatidylcholine liposomes by high-density lipoproteins and ascribed this process to a transfer of apolipoprotein from the lipoprotein to the liposome with the subsequent disruption of the latter. We confirm and extend their observation on the instability of liposomes in presence of high-density lipoprotein or plasma and offer an alternative interpretation of the results. Whatever is the mechanism by which the lipoprotein attacks the liposome, it is obvious that these observations should be of interest with respect to the widely advocated use of liposomes as intravenous carriers of drugs and similar substances [3]. Comparable observations have also been reported by Krupp et al. [4] in an investigation of cholesterol esterase activity on phosphatidylcholine/cholesterol vesicles.

Experimental

Multilamellar liposomes and single-bilayer lipid vesicles were prepared in 0.15 M NaCl/5 mM Tris · HCl, pH 7.4 (NaCl/Tris) from phosphatidyl choline as described before [1]. Either [³H]choline [1] or [¹⁴C]-linoleic acid [5] labeled rat-liver phosphatidylcholines diluted with unlabeled egg-yolk phosphatidylcholine were used. In some experiments [*Me*-¹⁴C]-labeled egg phosphatidylcholine was used, prepared by Dr. J.C. Wilschut according to the method of Stoffel [6]. Liposomes were separated from vesicles on a Sepharose 4B column (2 × 30 cm) or a Bio-Gel A-15m column (2 × 50 cm) eluted with NaCl/Tris. Column fractions were concentrated by means of ultrafiltration through Amicon PM-10 filters.

For incubation aliquots of liposomes or vesicles were mixed with an equal or double volume of plasma from rat, monkey or man or with a solution of purified monkey high-density lipoproteins (1 mg/ml). Both the monkey plasma and the lipoprotein were obtained from African green monkeys and were gifts from Dr. Lawrence Rudel from the Arteriosclerosis Research Center of the Bowman Gray School of Medicine. Incubations were done in a shaking water bath at 37°C for 30 or 120 min. Liposomes or vesicles were separated from the plasma proteins by gel filtration on an Ultrogel AcA34 column (3 × 50 cm), eluted with NaCl/Tris.

¹²⁵I-labeled albumin was prepared according to Bocci [7]. Column fractions

were assayed for ^{14}C and ^3H radioactivity by a liquid scintillation method with Omnifluor/Triton X-100 [4] as a scintillation mixture. ^{125}I -radioactivity was measured with a Nuclear Chicago γ -counter.

Protein was assayed according to Lowry et al. [8] with bovine serum albumin as a standard. Phospholipid phosphorus was determined according to Ames and Dubin [9]. Flotation of lipoproteins by ultracentrifugation in concentrated KBr solutions was carried out in a SW-41 rotor of a Beckman Spinco ultracentrifuge as described elsewhere [5].

Immunoelectrophoresis was done on 1% agarose gels on microscope slides according to Scheidegger [10]. The gels were run for 2 h at 10 mA in a 0.05 M barbital buffer, pH 8.6. Serum albumin, stained with bromophenol blue, was used as a marker. After electrophoresis, rabbit antiserum against whole monkey serum and rabbit antiserum against human high-density lipoprotein, which was prepared and made available by Dr. Lawrence Rudel, were allowed to react with the separated lipoproteins during a 72-h period. After soaking for 72 h in 0.15 M NaCl, the gels were either cut into 5-mm pieces to be assayed for radioactivity or used for autoradiography (exposure time 90 h).

Results

When liposomes consisting of radioactive phosphatidylcholine and obtained from the void volume of a Sepharose 4B or a Bio-Gel A-15m column were incubated with rat plasma and rechromatographed after incubation on the same column, two radioactivity peaks appeared, one in the void volume corresponding to the position of the liposomes and another in an elution volume somewhat smaller than that in which the main plasma protein peak eluted (not shown). Since the major constituent of this protein peak was albumin, it was obvious that most of the second radioactivity peak was not associated with albumin, in spite of our earlier observations that this protein alone is capable of taking up phosphatidylcholine from liposomes [1].

Complete separation of the second radioactivity peak from albumin and the other major protein constituents of the plasma was achieved on an Ultrogel AcA34 column (Fig. 1). An incubation mixture of multilamellar liposomes and rat plasma was first chromatographed on a Bio-Gel column to separate remaining liposomes from the plasma proteins. The fractions constituting the second radioactivity peak were pooled, concentrated approximately 10-fold and rechromatographed on the Ultrogel column. The elution pattern obtained is shown in Fig. 1. As a result of the pre-run on the Bio-Gel column, no liposomes are remaining, indicated by the absence of radioactivity in the void volume. Radioactivity is concentrated mainly in a peak eluting well ahead of the bulk of the protein. This major radioactivity peak consisted more than 90% of phosphatidylcholine. The second radioactivity peak, representing approximately 10% of the total radioactivity, consisted of free fatty acid only, as was shown by thin-layer chromatography. It is most likely bound to albumin, which elutes from the column at the position indicated by the arrow in Fig. 1. Apparently some hydrolysis of the phospholipid, which was labeled at the 2-position with radioactive linoleic acid, occurred during experimental procedures, either spontaneously or as a result of plasma phospholipase activity [11]. No appreciable

Fig. 1. Chromatography of radioactive phosphatidylcholine on an Ultrogel AcA34 column. 6. nm (●) — the Ultrogel elute as well as authentic m

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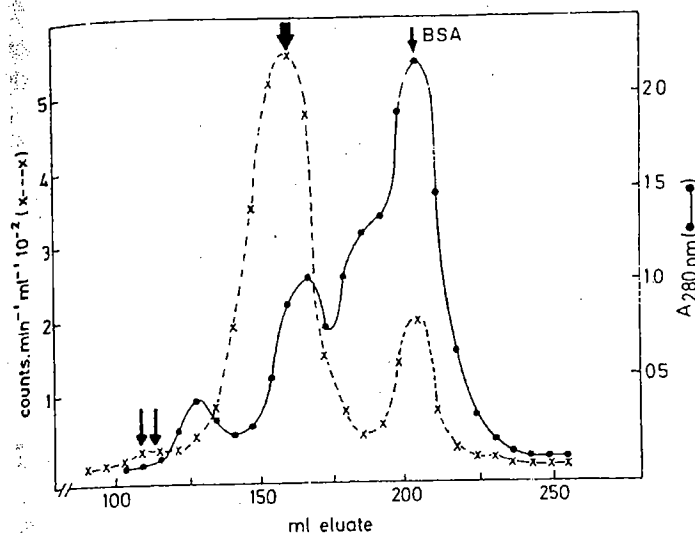


Fig. 1. Chromatography on Ultrogel AcA34 of plasma proteins labeled with radioactive phosphatidylcholine during incubation with liposomes. 2.3 ml of liposomes (2.2 μ mol of egg phosphatidylcholine and 0.1 μ mol 14 C-labeled rat-liver phosphatidylcholine, $1.4 \cdot 10^5$ cpm) in NaCl/Tris eluting in the void volume of a Bio-gel A-15m column, were incubated for 2 h with 4.6 ml of rat plasma and subsequently rechromatographed on the same Bio-Gel column to remove residual liposomes. The fractions containing radioactive protein (20 000 cpm) were pooled, concentrated and rechromatographed on the Ultrogel column. 6.4-ml fractions were collected and assayed for radioactivity (X---X) and absorbance at 280 nm (●—●). The single arrow (BSA) indicates the position at which bovine serum albumin elutes from the Ultrogel. The double arrow designates the void-volume in which both uni- and multilamellar liposomes elute as well as the (very)low-density lipoproteins. The single thick arrow indicates the elution volume of authentic monkey high-density lipoprotein.

formation of radioactive cholesteroesters was observed indicating that the liposomal phosphatidylcholine did not serve as a substrate for phosphatidylcholine : cholesterol acyltransferase under these conditions.

The position of the main radioactivity peak suggested that the lipid became associated with plasma components of fairly high molecular weight. Binding to globulins was excluded on the basis of complete lack of binding of liposomal phosphatidylcholine to commercial γ -globulin fractions (not shown). Our tentative assumption that liposomal phosphatidylcholine was transferred to lipoproteins was supported by the observation that a sample of authentic monkey high-density lipoproteins eluted from the Ultrogel column in the same volume as the main radioactivity peak. We set out to obtain further evidence of the involvement of high-density lipoprotein in the transformation of the liposomes.

Single-bilayer vesicles prepared from radioactive phosphatidylcholine were incubated with rat plasma and part of the incubation mixture was chromatographed on Ultrogel to estimate the amount of phospholipid released from the vesicles. 75% of the phosphatidylcholine appeared to be associated with the vesicles. 15% of the phosphatidylcholine appeared to be associated with the peak eluting at the elution volume of high-density lipoprotein; approx. 10% remained in the void volume. The remainder of the incubation mixture was used to demonstrate the lipoprotein character of the phospholipid-binding component by flotation of the lipoproteins after adjusting the density with KBr to 1.23. After centrifugation, the upper layer containing the lipoproteins

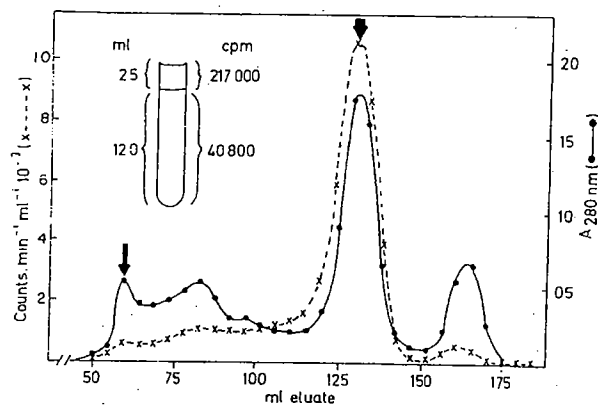


Fig. 2. Density gradient flotation of lipoproteins and their subsequent separation on Bio-Gel A-15m. Lipid vesicles were prepared from ^{14}C -labeled rat liver phosphatidylcholine and unlabeled egg phosphatidylcholine (molar ratio 1 : 185) and separated from multilamellar liposomes on Bio-Gel. 7 ml of vesicles in NaCl/Tris (2.5 μmol of lipid, $3.2 \cdot 10^5$ cpm) were incubated with 7 ml of rat plasma. After 2 h at 37°C , KBr was added to 11 ml of the incubation mixture to adjust density to 1.230. The solution was transferred to a 14-ml centrifuge tube and was filled up by layering NaCl/Tris containing KBr (density 1.225) on top of the incubation mixture. The tube was centrifuged for 40 h at 41 000 rev/min. After the run the upper layer was removed, assayed for radioactivity and chromatographed on Bio-Gel. 3.4-ml fractions were collected and assayed for radioactivity (X---X) and absorbance at 280 nm (●---●). The long arrow indicates the void volume of this column, in which multilamellar liposomes elute. The short, thick arrow indicates the position at which high-density lipoprotein elutes. In the elution volume between 80 and 120 ml low-density lipoproteins as well as unilamellar vesicles elute.

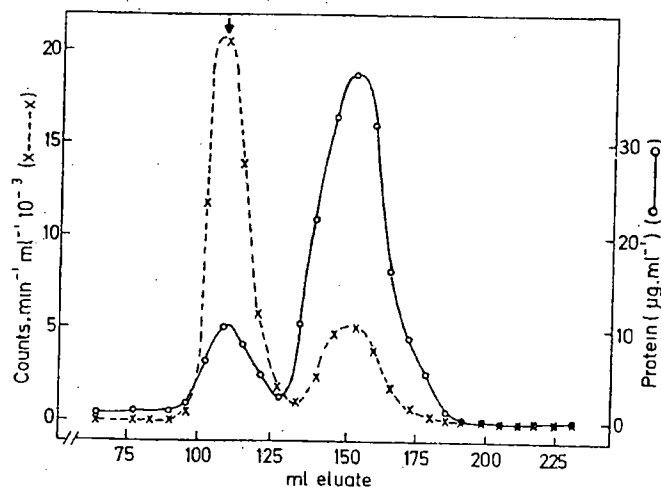


Fig. 3. Incubation of phosphatidylcholine vesicles with purified high-density lipoproteins and subsequent chromatography on Ultrogel AcA34. Single-bilayer vesicles were prepared from ^{14}C -labeled rat-liver phosphatidylcholine and unlabeled egg phosphatidylcholine (molar ratio 1 : 100) on a Bio-Gel A-15m column. 2 μmol of vesicles ($2 \cdot 10^5$ cpm) in 3.0 ml NaCl/Tris were incubated for 30 min with purified monkey high-density lipoprotein (1.3 mg of protein) and chromatographed on Ultrogel AcA34. 6.4-ml fractions were collected and assayed for radioactivity (X---X) and protein (○---○). Protein readings were corrected for blanks, containing the appropriate amounts of phospholipid. The arrow marks the void volume of the column in which remaining liposomes elute. Authentic high-density lipoprotein elutes between 140 and 170 ml.

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together with the 15% remaining vesicles was removed, assayed for radioactivity and transferred to the Bio-Gel column. Fig. 2. shows the elution pattern of protein and radioactivity from the Bio-Gel column as well as the distribution of radioactivity in the centrifuge tube. More than 85% of the radioactivity floated in the upper part of the centrifuge tube. Upon gel filtration of this upper layer on Bio-Gel the bulk of the radioactivity was found to elute with the high-density lipoprotein peak, to some extent contaminated with the small proportion of intact vesicles, as on this column such vesicles eluted at 110 ml. In addition to the radioactive lipoprotein peak we observed radioactivity eluting at smaller volumes, possibly representing low-density lipoproteins [12] which might also take up some of the liposomal phospholipid. The protein peak at approx. 165 ml probably represents some albumin inadvertently being removed with the upper layer of the gradient and containing some radioactive free fatty acid. During incubation of pure monkey high-density lipoproteins with liposomes a similar transfer was observed of phosphatidylcholine from liposomes to a compound with characteristics of high-density lipoprotein. An example of such an experiment, in which the incubation mixture was chromatographed on Ultrogel is shown in Fig. 3. Under these conditions, with high-density lipoprotein being the only protein component present, it is apparent that not only did release of phosphatidylcholine take place from liposomes to lipoprotein but that protein was transferred from the lipoprotein to the lipid vesicles as well, as indicated by the presence of protein in the void volume.

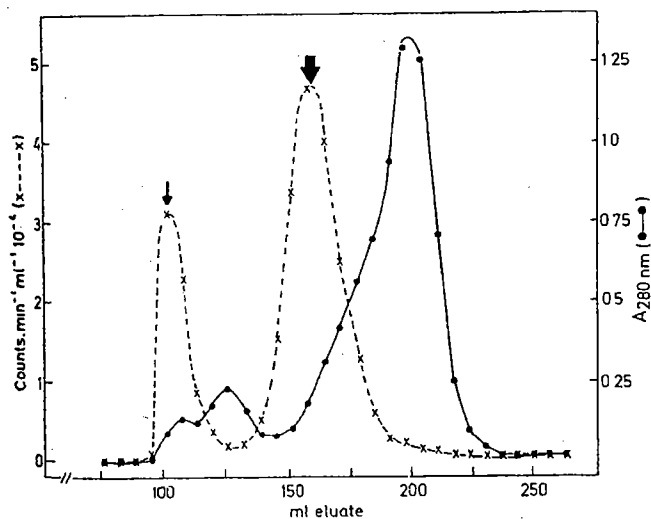


Fig. 4. Elution pattern on Ultrogel AcA34 of an incubation of labeled liposomes and monkey plasma. 1 ml sonicated, unfractionated liposomes prepared from 0.2 μ mol 14 C-labeled rat-liver phosphatidylcholine ($3.3 \cdot 10^6$ cpm) were incubated with 1 ml plasma from African green monkey. After 30 min 1.8 ml of the mixture was chromatographed on Ultrogel AcA34 and 6.4-ml column fractions were assayed for radioactivity (X---X) and absorbance at 280 nm (●—●). The long arrow marks the void volume in which any remaining multilamellar liposomes or unilamellar vesicles elute, as well as (very) low-density lipoproteins. The short, thick arrow indicates the volume in which high-density lipoproteins elute from this column. The main protein peak at approx. 200 ml represents mostly albumin.

The question, of course, arises as to whether the observed loss of phosphatidylcholine from liposomes or vesicles reflects an exchange diffusion mechanism or a form of net transport.

Therefore, we measured the specific radioactivities of the phosphatidylcholine remaining in the liposomes after incubation with plasma. In case the phospholipid released is entirely replaced by unlabeled phospholipid from the lipoproteins (exchange diffusion) the specific radioactivity of the liposomal phospholipid is expected to decrease proportionally with the extent of transfer. If, on the other hand, the phospholipid transfer is a one-way phenomenon, i.e., release from liposomes without concomitant replacement by lipoprotein phospholipid, we expect the specific radioactivity to remain constant.

In two such experiments with different liposomal preparations we found the specific radioactivity of the liposomes to change from 1580 and 1170 dpm/nmol, respectively, before incubation to 1635 and 1120 dpm/nmol, respectively, after a 30-min incubation in 50% plasma. The absolute amounts of phospholipid added to the incubation mixtures were 0.6 and 1.5 μmol respectively, of which, during incubation, 63 and 42%, respectively, were transferred to the lipoprotein particle. These observations show that the specific radioactivity, despite a considerable loss of radioactivity from the liposomes, does not

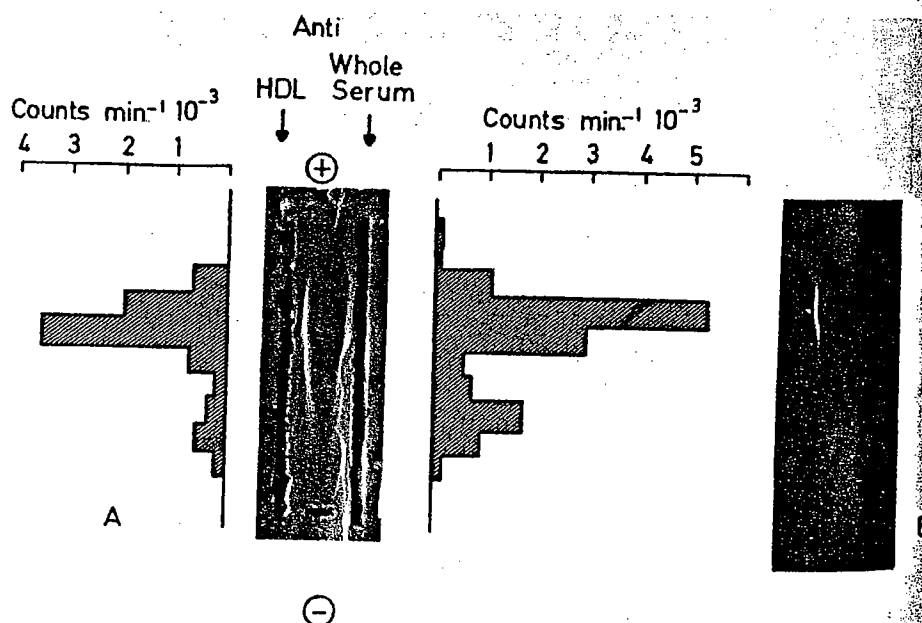


Fig. 5. Immuno-electrophoresis of an incubation mixture of labeled liposomes with monkey plasma. Approx. 20- μl samples of the incubation mixture described in the legend to Fig. 4 were subjected to microelectrophoresis on an agarose gel as described under Experimental. After electrophoresis precipitation lines were allowed to form with antiserum against whole monkey serum (right part of slide) and antiserum against human high-density lipoprotein (HDL) (left part of slide). Left- and right-hand sides of one gel were cut into 5-mm pieces which were assayed for radioactivity. In Fig. 5A the distribution of radioactivity along the gel is shown. Fig. 5B shows an autoradiogram of another, similar gel.

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change, indicating that, indeed, lipid is transferred from liposomes only and not vice versa.

The identity of the main phosphatidylcholine-binding complex was further investigated by immunoelectrophoresis. After incubation of liposomes, prepared from phosphatidylcholine of high specific radioactivity, with monkey plasma the bulk of the mixture was subjected to gel filtration on Ultrogel to assess the extent of lipid release from the liposomes. The elution pattern, which was essentially similar to those obtained after incubation with rat plasma, is shown in Fig. 4. Approx. 70% of the radioactivity was associated with the high-density lipoprotein peak. The remainder, representing residual liposomes and (very) low-density lipoproteins, was recovered in the void volume. 20- μ l aliquots of the incubation mixture were subjected to microelectrophoresis on agarose gels. Subsequent interaction with rabbit antiserum against whole monkey serum or rabbit antiserum against human high-density lipoprotein revealed precipitation lines as shown in Fig. 5A. This figure also shows the radioactivity distribution along the direction of electrophoresis at the left-hand and right-hand side of the gel. The bulk of the radioactivity was recovered in the area corresponding to the high-density lipoprotein line. From another gel obtained from the same incubation mixture an autoradiogram is shown in Fig. 5B. The main precipitation line at the right-hand side of the gel (the side at which the whole-serum antiserum was added) is that of the high-density lipoprotein as is apparent upon comparison with the left-hand side of the gel. A faint precipitation line is observed closer to the origin, presumably representing low-density lipoprotein. This observation is consistent with the elution pattern shown in

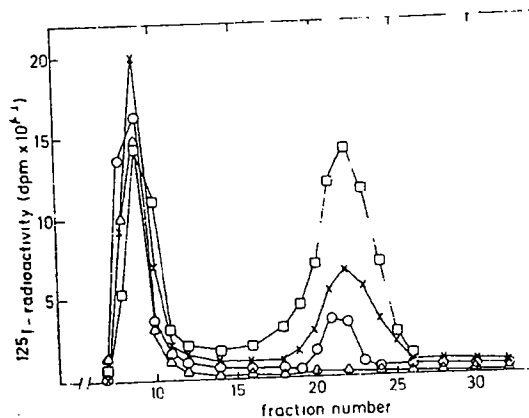


Fig. 6. Liposomes were prepared from egg phosphatidylcholine, cholesterol and dicetylphosphate (molar ratio 7 : 1 : 2) in presence of ^{125}I -labeled albumin (specific radioactivity 6000 cpm/ μg). Non-entrapped albumin was removed by gel filtration on Sepharose 4B. The void-volume fractions containing the bulk of albumin were combined and contained 2.5 μmol P and 180 000 cpm/ml. The liposomes were stored at 4°C under N_2 . 1.0 ml of this [^{125}I]albumin-containing liposome preparation was either mixed with 1.0 ml freshly isolated heparinized rat plasma and incubated for 30 min at 37°C followed by rechromatography on the Sepharose column or mixed with 1.0 ml ice-cold plasma and immediately rechromatography on the Sepharose. Fractions of 4.0 ml were collected and assayed for ^{125}I -radioactivity. Graphed on the Sepharose. Fractions of 4.0 ml were collected and assayed for ^{125}I -radioactivity. Legend: \circ — \circ , control without plasma, immediately after preparation; \triangle — \triangle , control without plasma; 5 days after preparation; \times — \times , with plasma, without incubation; 1 day after preparation; \square — \square , with plasma, after 30 min incubation at 37°C, 2 days after preparation.

Fig. 2, which also revealed radioactivity associated with larger lipoproteins. The diffuse spot on the autoradiogram is caused by remaining vesicles: from Fig. 4 it was clear that some residual liposomal radioactivity had to be expected. Nearly identical results were obtained when human rather than monkey plasma was used in the incubation with the lipid vesicles.

In a previous publication [1] we suggested a relationship between sucrose or inulin release from liposomes and the transfer of liposomal phosphatidylcholine to plasma components. Since the components we used for leakage studies were of relatively low molecular weight and uncharged it might be argued that liposomal integrity is grossly retained during incubation with blood or plasma and that leakage occurs through small (transient) "holes" in the liposomal membranes. The massive one-way release of phosphatidylcholine from the liposomes of which we obtained evidence in the present study is difficult to reconcile with such a view. Release of liposome-entrapped ^{125}I -labeled albumin from the liposomes as a result of incubation with plasma is shown in Fig. 6 and supports our conviction that the interaction of (high-density) lipoproteins with the liposome eventually leads to desintegration of the liposomal structure. Incubation for 30 min at 37°C released about 60% of the trapped albumin. Also at low temperature (4°C) considerable release took place, presumably during the time required for the sample to penetrate into the column bed. Spontaneous albumin release could not be avoided during storage, but it is obvious from Fig. 6 that most of the protein release in presence of plasma was caused by the incubation. Although some adsorption of albumin to the liposomes can not be excluded, we believe that the bulk of the liposome-associated albumin is actually entrapped, because with this protein we find roughly the same trapping efficiency as for sucrose, inulin and horse-radish peroxidase, while, in addition, the latter enzyme is nearly completely latent in absence of detergents. The bulk of the released albumin, representing more than 60% of the total liposome-associated protein, must, therefore, have been entrapped in the aqueous compartments.

Discussion

The extensive release of phosphatidylcholine from liposomes during incubation with plasma with the concomitant association of the phospholipid with a lipoprotein-like particle, as demonstrated in this paper, is likely to be the cause of the sucrose, inulin and albumin leakage from liposomes which we observed previously [1] and in this study. The observation that the specific radioactivity of the liposome-associated phosphatidylcholine is not significantly lowered after transfer of a considerable proportion of the phospholipid supports this view, as it proves that no appreciable amount of unlabeled phosphatidylcholine is transported from the lipoproteins to the liposomes. Therefore, massive destruction of the liposomal structure must occur as a consequence of the loss of the bulk of its phospholipid. The release of phospholipid from the liposomes apparently involves an interaction of liposome and high-density lipoprotein, as is indicated by our experiments with the pure lipoprotein. Phospholipid transfer proteins such as described in a variety of tissues by Wirtz and several other workers (see ref. 13 for a review) are apparently not involved. Also albumin,

which we demonstrated to bind liposomal phosphatidylcholine in a reversible way, is not required for the transfer to take place.

The results described in this paper demonstrate that the phosphatidylcholine released from the liposomes during incubation with plasma becomes associated with a particle which shares several properties with high-density lipoproteins: its behaviour on gel filtration indicates a similar molecular weight, its electrophoretic properties on agarose are those of high-density lipoprotein and it is precipitated by antibodies against high-density lipoproteins. The most obvious conclusion would be, therefore, that liposomal phospholipid during interaction with the plasma high-density lipoprotein is transferred to the latter. The thus additionally acquired phospholipid would not alter the molecular weight of the lipoprotein sufficiently to change its elution properties on Ultrogel and, in addition, it would probably not alter the immunoelectrophoretic properties of the particle, those being dictated predominantly by the protein moiety. However, our observations are also compatible with an alternative interpretation, as recently proposed by Tall and Small [2]. These authors suggested, based mainly upon analogy with results obtained with apolipoprotein A-I, that the high-density lipoprotein releases apolipoprotein A-I which becomes associated with the liposomes and subsequently "dissolves" those to form a phosphatidylcholine · apolipoprotein A-I complex. Although solid evidence for this interpretation was not presented, it is an interesting hypothesis which stands firmly against the view that liposomal phosphatidylcholine is transferred from the liposomes to the whole high-density lipoprotein particle. Our finding that the liposomal void-volume peak, after incubation with plasma, contained protein could be considered as compatible with a transfer of apolipoprotein to the liposomes. However, more detailed experimental evidence is required to decide which of these two possibilities reflects the actual mechanism of liposome disruption. We are currently investigating this problem in our laboratory. The outcome of such experiments will be important for the explanation of our recent observations on the clearance from the blood and uptake by the liver of intravenously injected liposomal phosphatidylcholine [14]. We found that such phosphatidylcholine is taken up to large extents by the parenchymal cells of the liver, in contrast to liposome-entrapped proteins. Possibly this uptake of phosphatidylcholine by the hepatocytes represents the fate of the phosphatidylcholine · (lipo)protein complex formed as a result of the interaction of liposomes with high-density lipoproteins. The half-life we found for the phosphatidylcholine in this complex during liver perfusion or in vivo was approx. 0.5 h and 1 h, respectively. This suggests at least that the uptake of the lipid from the circulation does not represent the uptake of high-density lipoprotein as such since Roheim et al. [15] reported a half-life of 10.5 h for the protein moiety of this lipoprotein in rat. The lipid is apparently cleared separately, either from a modified high-density lipoprotein particle or from a newly formed phosphatidylcholine · apolipoprotein complex.

We have not thoroughly investigated the physical nature of the phospholipid which is recovered in the void volume of the Sepharose 4B or Bio-gel A-15m columns after incubation of liposomes with plasma. Our observation that liposome-entrapped albumin remains associated with the void volume to approximately the same extent as the liposomal phospholipid (roughly one-

third under the conditions applied) argues in favor of the assumption that this phospholipid constitutes non-degraded liposomes which acquired some protein material from lipoproteins or other proteins, as was recently reported by Tyrrell et al. [16]. Electron microscopy revealed the presence of liposomal structures in this fraction indicating that at least part of the lipid remains liposome-bound. It has been suggested [4] that the relatively low rate of clearance from the blood of intravenously injected unilamellar phosphatidylcholine vesicles as observed by Juliano and Stamp [17] can be explained by the effectiveness with which such vesicles desintegrate as a result of their interaction with high-density lipoproteins. We have confirmed the reported instability of unilamellar lipid vesicles and substantiated the identity of the plasma component responsible for this instability. We also demonstrated that multilamellar liposomes are susceptible to lipoprotein-induced damage as well. Probably the larger number of lamellae per particle allow more time for the cells of the reticulo-endothelial system, e.g., Kupffer cells of the liver, to phagocytize a substantial proportion of the particulate liposome-associated phosphatidylcholine before complete disruption of the liposome has been accomplished. Once the phosphatidylcholine becomes lipoprotein-associated it will probably not be taken up by the Kupffer cells but rather, much more slowly, by the parenchymal cells of the liver. The half-life we found for such lipoprotein-associated phosphatidylcholine during liver perfusion or in vivo compares favorably with the values found for the half-life of intravenously injected unilamellar vesicles [17].

The many investigators who reported uptake of intravenously administered liposome-entrapped substances during recent years invariably used phosphatidylcholine-containing liposomes. In our opinion there is no doubt that substantial damage must have occurred to such liposomes resulting in leakage of the entrapped compounds and thus obscuring the actual fate of the liposome-associated material. The amount of lipid injected as well as detailed liposomal composition may very well influence the extent of damage inflicted upon the liposomes, thus giving rise to variable results by different investigators. Experiments designed to obtain liposomal preparations of maximal stability are currently in progress in our laboratories.

Very recently two reports appeared in which evidence was presented of the formation of a modified high-density lipoprotein particle as a result of uptake of additional phosphatidylcholine from liposomes. Yokoyama et al. [18] demonstrated that Sepharose-bound high-density lipoprotein was able to bind phosphatidylcholine from sonicated dispersions. Forte et al. [19] found that upon incubation of phosphatidylcholine liposomes with high-density lipoproteins two particles were formed, one resembling the particle proposed by Tall and Small [2] and another representing the high-density lipoprotein particle containing some additional liposome-derived phosphatidylcholine.

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